Review of: "An essential vesicular-trafficking phospholipase mediates neutral lipid synthesis and contributes to hemozoin formation in Plasmodium falciparum"

Kildare Miranda[1], Camila Wendt

1 Universidade Federal do Rio de Janeiro

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Functional role of lipid metabolism in hemozoin nucleation

Malaria remains as a major health problem, being responsible for more than 200 million cases and over 400 thousand deaths in 2019 [1]. Significant efforts have been made to develop effective prevention strategies and to improve the effectiveness of antimalarial drugs currently used. However, achieving effective intervention strategies depends on a deep knowledge on parasite biology, making the study of a model as complex as *Plasmodium* a fertile ground for the discovery of novel host-parasite interaction mechanisms. As the main clinical aspects of the disease in humans are directedly related to parasite development inside erythrocytes [2], understanding the mechanisms by which the parasite modulates host cell structure and metabolism is crucial. Part of these mechanisms involves the assembly of an elaborated exomembrane system [3, 4] and the uptake of large amounts of hemoglobin from host cell cytoplasm [5–7]. Degradation of internalized hemoglobin inside the food vacuole, releasing aminoacids and conversion of free heme into hemozoin crystals or ‘malarial pigment’ are amongst the most relevant topics on malaria biology [8].

Hemozoin is a unique structure so far described only in malaria parasites and blood-feeding organisms [9–12]. This crystal plays multiple roles on malaria biology, being used for more than a century as a biomarker for malaria detection in blood smears. Hemozoin may potentially modulate the host immune system [9, 13, 14] and has been shown to remain in the host body for long periods of time [15]. Crystallization mechanisms also stand as important drug targets, since hemozoin synthesis is vital for parasite development inside erythrocytes. In this regard, pharmacological inhibition of hemoglobin catabolism and hemozoin nucleation have proven efficient methods to clear *Plasmodium* spp. infection in the vertebrate host [8, 10]. Resistance to antimalarial drugs, however, remains as a major problem. Understanding the molecular, biochemical and structural basis of such drug targets is therefore of paramount importance for the development of more efficient therapies.

Many groups have worked on the characterization of hemozoin crystal structure and its synthetic analogue, β-hematin [16–20]. Yet, the precise mechanisms underlying hemozoin formation remains elusive. Nevertheless, it is widely recognized that a nucleation center is required to initiate the process, although different hypothesis have been postulated manly based on the role of proteins [21–23] or lipids [24–27] to promote heme dimerization and crystal propagation.

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n this regard, the recent work developed by Asad *et al.*, comes at the right time, shedding light on the importance of lipid metabolism for hemozoin formation in *Plasmodium falciparum*. In their recent work, the authors characterized the functional role of the parasite lysophospholipase, PfLPL1, on the synthesis of neutral lipids and its importance for hemozoin nucleation during its intra erythrocytic development. Fluorescence microscopy and transmission electron microscopy approaches revealed that PfLPL1 containing vesicles traffic from parasite periphery towards vicinity of food vacuole, potentially contributing to the regulation of hemozoin formation. Knock-down of PfLPL1 resulted in the reduction of food vacuole associated neutral lipids and disrupted hemozoin formation within the parasite. The phenotype observed in knocked down parasites was very interesting and corroborates previous observations that suggested the importance of lipids on hemozoin formation. It is an important finding, since previous attempts to disturb proteins considered to be involved on hemozoin nucleation process were not that effective. *P. falciparum* mutants that lack histidine-rich protein II (HRPII), for example, showed no perturbation to hemozoin growth or heme crystallization [28]. Molecular attempts to disturb another proteins relate to hemozoin formation, heme detoxification protein (HDP), have so far been unsuccessful [21].

Asad *et al.*, work is interesting and definitely worth of reading for the malaria community. In addition to the important contribution to the understanding of lipid metabolism on malaria parasites, it provides a clear link between lipid catabolism and hemozoin nucleation pathway. Recent works have shown that phospholipases are essential for *P. falciparum* transition from the asexual blood stages to gametocytes [29], for gametogenesis fertilization [30] and for schizogonic asexual division [31], highlighting the importance of membrane remodeling by phospholipase for parasite development and the contribution of the findings by Asad et al., for malaria biology. Phospholipases are encoded by 22 different genes in *P. falciparum* [32] and their enzymatic activities, location and expression patterns are nevertheless not well characterized. Further studies on the role of phospholipases will definitely provide novel information on lipid metabolism in malaria parasites, perhaps bringing new insights on parasite survival mechanisms.

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